

## Purification of Semaglutide and Liraglutide on NanoPak-C All Carbon HPLC columns

**Background.** Semaglutide and liraglutide are peptide drugs to treat diabetes and obesity. They are glucagon-like peptide-1 (GLP-1) analogs that mimic GLP-1 receptor agonists, a natural hormone that regulates blood sugar levels. Separation and purification of these peptides are essential to meet drug quality requirements.

Optimizing their separation and purification process involves selecting appropriate media, mobile phase composition, pH, flow rate, and temperature. NanoPak-C, All Carbon columns' features, include pH and temperature stability and tunable properties (selectivity, pore size, surface area). These features improve the separation and purification performance and reduce operational and maintenance costs. The NanoPak-C (40um particle size) columns can be used as a cost-effective, robust, and efficient solution for pre-purifying peptides such as Semaglutide and Liraglutide.

This application note outlines a general approach to developing a simple and robust high-performance liquid chromatography (HPLC) method with 40um particle size column and diode array detection for the analysis of Semaglutide and Liraglutide.

### Probe Analytes

**Semaglutide:** BOC Sciences. CAS No. 910463-68-2  
1 mg/mL in DI water pH 11.1 (20mM NH<sub>4</sub>OH).

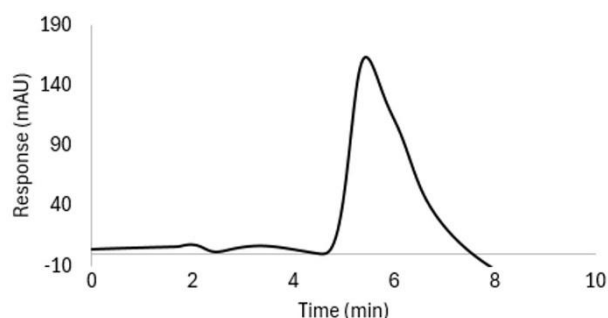
**Liraglutide:** BOC Sciences. CAS No. 204656-20-2.  
1 mg/mL in DI water pH 11.1 (20mM NH<sub>4</sub>OH).

### Instrumentation

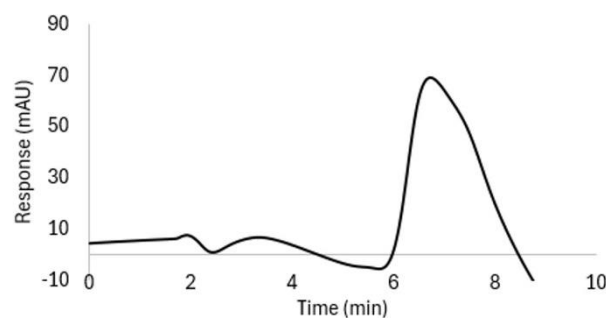
<b>HPLC Conditions</b>									
<b>Methods</b>									
<b>Column</b>	Nanopak-C All Carbon 250 x 4.6 mm, 40 um								
<b>Mobile phase (Acidic)</b>	Mobile Phase A: 20 mM HCOONH <sub>4</sub> -NH <sub>3</sub> (pH 8.5) Mobile Phase B: Acetonitrile Gradient: <table border="1" style="margin-left: 20px;"> <thead> <tr> <th>Time</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>20</td> </tr> <tr> <td>10</td> <td>65</td> </tr> <tr> <td>11.5</td> <td>20</td> </tr> </tbody> </table>	Time	%B	0	20	10	65	11.5	20
Time	%B								
0	20								
10	65								
11.5	20								
<b>Injection volume</b>	20ul								
<b>UV detection</b>	220nm								
<b>Oven temperature</b>	25deg C								

### Results

**Figures 1 and 2** show representative chromatograms of Semaglutide and liraglutide, respectively. The results indicate that these columns can be used as a standalone cleanup method for Semaglutide and Liraglutide or as a pre-purification step for initially reducing sample complexity and removing a significant portion of impurities. This cleanup can be followed by final purification using preparative HPLC columns with 5-10um media.



**Figure 1. Representative chromatogram of Semaglutide.**



**Figure 2. Representative chromatogram of Liraglutide.**